EPA/OPP MICROBIOLOGY LABORATORY
ESC, Ft. Meade, MD

Standard Operating Procedure for Use of the AOAC Use Dilution Test and the Germicidal Spray Products Test without Test Microbes to Determine the Presence of Microbial Contamination in EPA-Registered Hospital Disinfectants

SOP Number: QC-21-00

Date Revised: 1-18-06

Initiated By: _____ Date: ___/___/___

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Effective Date: ___/___/___

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1.0 SCOPE AND APPLICATION:

1.1 The assay provides data on the occurrence of contamination when an antimicrobial product is evaluated using the AOAC Use Dilution Test or the AOAC Germicidal Spray Products Test. The assay is qualitative and designed to recover and culture bacterial contamination (i.e., viable spores of *Bacillus* sp.) from a hospital disinfectant sample. In most instances, the assay will be deemed necessary due to historical evidence of bacterial contamination associated with the sample itself, a previously-collected sample of the same product, or a registrant's product line. Products that show no evidence of product-borne contamination when tested by this procedure are considered adequate for efficacy testing; however, samples with confirmed contamination should not be used for conducting efficacy tests

2.0 DEFINITIONS:

2.1 AOAC = AOAC International

3.0 HEALTH AND SAFETY:

3.1 Disinfectants may contain a number of different active ingredients, such as heavy metals, aldehydes, peroxides, phenol, etc. Latex gloves and other personal protective clothing or devices are worn during the handling of these items for purpose of activation or dilution. A chemical fume hood or other containment equipment is employed when performing tasks with concentrated products.

4.0 CAUTIONS:

4.1 Follow appropriate chain-of-custody guidelines during testing as stipulated in SOP-COC-01, Sample Log-in and Tracking. Sample handling and custody procedures are considered the same as for efficacy testing.

4.2 To avoid introduction of contaminating organisms into the test system and cross-contamination from tube to tube during the assay, it is recommended that hooks be autoclaved prior to use, that one hook be used for a predetermined number of tubes and then be replaced with an autoclaved, unused hook, that there is sufficient time in between transfers (from disinfectant to neutralizer and neutralizer to subculture medium) to thoroughly flame (to red-hot) the wire hook, and that the use of a water bath be avoided (see section 10.1.3). In addition, the ultraviolet light (UV) in the biological safety cabinet should be left on overnight prior to performing the assay (see section 10.1.4).

5.0 INTERFERENCES:

5.1 Aseptic procedures must be followed during this assay to avoid accidental contamination of the product. Exposing the product to external contaminants during opening and dispensing, and the use of non-sterile laboratory supplies may interfere with the outcome of this analysis. Quality control measures for media and reagents used in this evaluation must be followed as outlined in SOP QC-11, Performance and Sterility of Media and Reagents.

6.0 PERSONNEL QUALIFICATIONS:

6.1 Personnel are required to be knowledgeable about the procedures in this SOP. Documentation of training and familiarization with this SOP can be found in the training file for each employee.

7.0 SPECIAL APPARATUS AND MATERIALS:

7.1 Carriers: polished stainless steel cylinders, 8 +/- 1 mm OD, 6 +/- mm ID, 10 +/- 1mm length; type 304 stainless steel, SS 18-8 (Fisher Scientific, catalog number 7-907-5)

7.2 Glass Slide Carriers, Bellco 25 mm × 25 mm (or comparable size) borosilicate glass cover slips with number 4 thickness (Bellco Glass, Inc., item number: 1916-S0131).

7.3 Appropriate Spray Disinfectant Apparatus

7.4 38 mm × 100 mm medication tubes (Bellco) for neutralization and subculture media for AOAC Germicidal Spray Products Test

7.5 VITEK System for the automated identification of microorganisms

7.6 Eppendorf Pipettes (1-10 μL)

7.7 PCS 2 Pipette Calibration System for Eppendorf pipettes

8.0 INSTRUMENT OR METHOD CALIBRATION:

8.1 Refer to the laboratory equipment calibration and maintenance SOPs (SOP EQ series) for details on method and frequency of calibration.

9.0 SAMPLE HANDLING AND STORAGE:

9.1 Disinfectants are stored according to manufacturers' recommendations or at room temperature if the product label or testing parameters do not identify a storage temperature.

10.0 PROCEDURE AND ANALYSIS:

10.1 To conduct the assay, follow OPP Microbiology Laboratory SOP MB-05, AOAC Use Dilution Method for Testing Disinfectants for liquid products, and SOP MB-06, Testing of Spray Products for spray products and liquid products to be applied as a spray. The following exceptions/comments will apply:

10.1.1 No inoculum will be generated as test microbes are not used in this study.

10.1.2 If test parameters call for an organic soil load, disregard this requirement. Because no inoculum is generated, no organic soil will be included. See section 10.1.7 of this SOP for a recommendation to apply an organic substance to glass slides in the event that the spray product beads up and rolls off of a glass slide in the Modified AOAC Germicidal Spray Products Test. The addition of an organic substance (e.g., 5% horse serum in sterile deionized water, letheen broth) in this case is to increase dispersion of the spray product across the entire slide.

10.1.3 For liquid products, the recirculating chiller and water bath specified in SOP MB-05 to maintain the temperature of the disinfectant will not be used. Elimination of the water bath will reduce any potential for introducing water-borne contaminants into the test system. Place racks of disinfectant-containing tubes directly onto the surface of the biological safety cabinet. Exposure of carriers to disinfectant will be performed at room temperature.

10.1.4 The ultraviolet light (UV) in the biological safety cabinet should be left on overnight prior to performing the test. Necessary test equipment (i.e., vortex, hooks, timer) will be placed into the biological safety cabinet prior to turning on the UV light.

10.1.5 A single assay involves the evaluation of 60 sterile carriers for one product sample. Approximately 65-70 sterile carriers (60 plus extras) will be prepared.

10.1.6 For liquid products: Sterile stainless steel carriers will be removed

from the asparagine solution, placed onto filter paper in sterile glass Petri dishes, and dried for 40 minutes per SOP MB-05. Once drying is complete, the modified AOAC Use Dilution Test will be conducted using the dried, uninoculated carriers according to the test parameters (i.e., product dilution, neutralizer, subculture media, contact time).

10.1.7 For spray products: Conduct the modified AOAC Germicidal Spray Products Test method using the sterile glass carriers according to the test parameters (i.e., product dilution, neutralizer, subculture media, contact time).

Recommendation: Before conducting the modified spray test, the analyst must practice applying the spray product to sterile glass slides to determine the product's level of dispersion. If the product beads up and rolls off of the slide rather than completely covering the glass slide as it would in a typical efficacy evaluation (with inoculated slides), the analyst should apply 10 ul of an organic material (e.g., 5% horse serum in sterile deionized water, letheen broth) onto the surface of each sterile glass slide and spread it with a sterile loop. Dry the slides for 40 minutes per SOP MB-06. Once drying is complete, apply the spray product again to determine if product dispersion is improved. Based on previous observations in our laboratory, the addition of an organic substance to the surface of the slide increases product dispersion on glass slides. If the application of an organic substance is deemed necessary to perform the test, apply the substance, dry the slides for 40 minutes as per SOP MB-06, and conduct the modified AOAC Germicidal Spray Products Test method using the dried, sterile carriers according to the test parameters (i.e., product dilution, neutralizer, subculture media, contact time).

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10.1.8 Carrier counts will not be performed.

10.1.9 Report results as (+) for microbial growth or (0) for no growth on the results sheet. In the "Comments" section of the results form, describe the growth in positive tubes (e.g., uniformly turbid, string-like, etc.). Note: Shaking tubes prior to recording results may disrupt the unique physical appearance of typical *Bacillus* sp. growing in liquid media (i.e., string-like, fibrous, not producing uniformly turbid media).

10.1.10 A minimum of three positive (showing microbial growth) carrier sets, if available, should be confirmed using Gram staining, growth on trypticase soy agar (TSA; for initial identification and isolation), and VITEK. If there are less than three positive carrier sets, then each carrier set will be confirmed. If both tubes are positive in a carrier set, only one tube is selected for confirmatory testing.

10.1.11 For a test with greater than 20 positive carrier sets, confirm at least 20% by Gram staining, TSA, and VITEK analysis. Again, if both tubes are positive in a carrier set, only one tube is selected for confirmatory testing.

11.0 DATA ANALYSIS/CALCULATIONS: None

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT:

12.1 Data will be recorded promptly, legibly, and in indelible ink on the appropriate forms. Completed forms are archived in notebooks kept in secured file cabinets in the file room D217. Only authorized personnel have access to the secured files. Archived data is subject to OPP's official retention schedule contained in SOP ADM-03, Records and Archives.

12.2 A performance report will be compiled as stipulated in SOP ADM-01, Performance Reports.

13.0 QUALITY CONTROL:

13.1 For quality control purposes, the required information is documented on the appropriate record form(s) (see 16.0).

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

14.1 If product is confirmed to be contaminated, no further testing will be initiated.

14.2 Strict adherence to the protocol is necessary for the validity of the test results. Any deviation from the standard protocol must be brought to the Study Director's attention and recorded in the raw data and an explanation for the deviation given. The deviation and reason for it must be documented on the GLP Compliance form in the final report.

15.0 REFERENCES:

15.1 Official Methods of Analysis. 1990. 15th Ed., Association of Official Analytical Chemists, Arlington, VA, (Method 955.14, 955.15, 961.02, and 964.02).

16.0 FORMS AND DATA SHEETS:

16.1 AOAC Use-Dilution Test without Test Microbes: Time Recording Sheet for Carrier Inoculation Steps

16.2 AOAC Use-Dilution Test without Test Microbes: Time Recording Sheet for Carrier Transfers

16.3 AOAC Use-Dilution Test without Test Microbes Information Sheet

16.4 AOAC Use-Dilution Test without Test Microbes Results Sheet

16.5 AOAC Germicidal Spray Test without Test Microbes: Time Recording Sheet for Carrier Inoculation Steps

16.6 AOAC Germicidal Spray Test without Test Microbes: Time Recording Sheet for Carrier Transfers

16.7 AOAC Germicidal Spray Test without Test Microbes: Information Sheet

16.8 AOAC Germicidal Spray Test without Test Microbes: Results Sheet

16.9 Test Microbe Confirmation Sheet

AOAC Use-Dilution Test without Test Microbes: Time Recording Sheet for Carrier Inoculation Steps

OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by: _____	
Test Date	
Type of Test	AOAC Use Dilution Test (UDT) without Test Microbes
Product Reg. No.	
Product Name	
Sample No(s).	
Organism	Not Applicable

Initials/Date	Test ID	Carrier Dry Time*	
		Start Time	End Time
	UDT		

* Recorded from laboratory clock/and timer.

AOAC Use-Dilution Test without Test Microbes: Time Recording Sheet for Carrier Transfers

OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by: _____	
Test Date	
Type of Test	AOAC Use Dilution Test (UDT) without Test Microbes
Product Reg. No.	
Product Name	
Sample No(s).	
Organism	Not Applicable

Initials/Date	Set	Drop Interval	Carrier Drop Start Time (into the disinfectant)		Carrier Drop End Time (into the primary subculture/neutralizer media)		Carrier Transfer (into secondary subculture)
			Clock	Timer	Clock	Timer	Start Time[1]
	1-20						
	21-40						
	41-60						
Comments:							

[1]Carrier transfer into secondary subculture (time elapsed after last carrier dropped in primary); taken from clock

AOAC Use-Dilution Test without Test Microbes Information Sheet
OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by:_____			
EPA Reg. No.		SOP	
Name		Test Date	
Sample No.		Comments: Assay will be performed without test microbes.	
Lot No.			
Expiration Date			

TEST PARAMETERS/Confirmed by:_____			
H_2O Hardness ($CaCO_3$) ppm	Specified	Titrated (Buret)/Date/Init.	HACH/Date/Init.
[redacted]			
Use Dilution	Specified	As Prepared/Date/Init.	
[redacted]			
Neutralizer	Specified		
[redacted]			
Contact Time	Specified	As Tested	
[redacted]			
Other Parameters	Specified		
[redacted]			

REAGENT/MEDIA INFORMATION/Confirmed by:_____			
Reagent/Media	Prep. No.	Reagent/Media	Prep. No.

AOAC Use-Dilution Test without Test Microbes Results Sheet
OPP Microbiology Laboratory

PRODUCT INFORMATION/Confirmed by:_____			
EPA Reg. No.		Test Date	
Name		Test Organism	Not Applicable
Sample No.			

CARRIER INFORMATION (to be completed by Analyst)		
Carrier Drop Time Interval	Carrier Set	Analyst

TEST RESULTS									
Date Recorded/Initials									
Primary Subculture / Secondary Subculture (carrier)									
1	2	3	4	5	6	7	8	9	10
/	/	/	/	/	/	/	/	/	/
11	12	13	14	15	16	17	18	19	20
/	/	/	/	/	/	/	/	/	/
21	22	23	24	25	26	27	28	29	30
/	/	/	/	/	/	/	/	/	/
31	32	33	34	35	36	37	38	39	40
/	/	/	/	/	/	/	/	/	/
41	42	43	44	45	46	47	48	49	50
/	/	/	/	/	/	/	/	/	/
51	52	53	54	55	56	57	58	59	60
/	/	/	/	/	/	/	/	/	/
Results Summary			Number of Carrier Sets with Growth						
			Number of Carrier Sets without Growth						
Modifications/Comments:									

AOAC Germicidal Spray Test without Test Microbes: Time Recording Sheet for Carrier Inoculation Steps

OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by:_____	
Test Date	
Type of Test	AOAC Germicidal Spray Products Test (GSPT) without Test Microbes
Product Reg. No.	
Product Name	
Sample No(s).	
Test Organism	Not Applicable

Initials/Date	Test ID	Carrier Dry Time*	
		Start Time	End Time
	GSPT		

* Recorded from laboratory clock/and timer.

AOAC Germicidal Spray Test without Test Microbes: Time Recording Sheet for Carrier Transfers

OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by:_____	
Test Date	
Type of Test	AOAC Germicidal Spray Products Test (GSPT) without Test Microbes
Product Reg. No.	
Product Name	
Sample No(s).	
Test Organism	Not Applicable

Initials/date	Set	Drop Interval	Carrier Spray Start Time (into the disinfectant)		Carrier Spray End Time (into the neutralizer/primary subculture)[1]		Carrier Transfer (into secondary subculture)
			Clock	Timer	Clock	Timer	Start Time[2]
	1-20						
	21-40						
	41-60						
Comments:							

[1]For spray test with *M. bovis*, the slide end time is when the slide is transferred into the neutralizer tube. The slide is then immediately transferred into MPB.
[2]Carrier transfer into secondary subculture; taken from clock

AOAC Germicidal Spray Test without Test Microbes: Information Sheet

OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by:_____			
EPA Reg. No.		SOP	
Name		Test Date	
Sample No.		Comments: Assay will be performed without test microbes.	
Lot No.			
Expiration Date			

TEST PARAMETERS/Confirmed by:_____			
H_2O Hardness ($CaCO_3$) ppm	Specified	Titrated (Buret)/Date/Init.	HACH/Date/Init.
[redacted]			
Use Dilution	Specified	As Prepared/Date/Init.	
[redacted]			
Neutralizer	Specified		
[redacted]			
Contact Time	Specified	As Tested	
[redacted]			
Other Parameters	Specified		
[redacted]			

REAGENT/MEDIA INFORMATION/Confirmed by:_____			
Reagent/Media	Prep. No.	Reagent/Media	Prep. No.

AOAC Germicidal Spray Test without Test Microbes: Results Sheet

OPP Microbiology Laboratory

PRODUCT INFORMATION/Confirmed by:_____			
EPA Reg. No.		Test Date	
Name		Test Organism	Not Applicable
Sample No.			

CARRIER INFORMATION (to be completed by Analyst)		
Carrier Spray Time Interval	Carrier Set	Analyst

TEST RESULTS									
Date Recorded/Initials									
Primary Subculture / Secondary Subculture (carrier)									
1	2	3	4	5	6	7	8	9	10
/	/	/	/	/	/	/	/	/	/
11	12	13	14	15	16	17	18	19	20
/	/	/	/	/	/	/	/	/	/
21	22	23	24	25	26	27	28	29	30
/	/	/	/	/	/	/	/	/	/
31	32	33	34	35	36	37	38	39	40
/	/	/	/	/	/	/	/	/	/
41	42	43	44	45	46	47	48	49	50
/	/	/	/	/	/	/	/	/	/
51	52	53	54	55	56	57	58	59	60
/	/	/	/	/	/	/	/	/	/
Results Summary			Number of Carrier Sets with Growth						
			Number of Carrier Sets without Growth						
Modifications/Comments:									

Test Microbe Confirmation Sheet
OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by:_____			
EPA Reg. No.		Test Date	
Name		Test Organism	Not Applicable
Sample No.		Comments	

Source: Tube/Plate ID	Date/ Initials	Stain Results*	Media Information			Results		
			Name	Prep. No.	Inc. Time/ Temp.	Date/ Initials	Colony Characteristics	VITEK ID** (if applicable)

* GPC=Gram positive cocci, GNR=Gram negative rods, GPR=Gram positive rods
** VITEK numerical profile number